18. (Amended) A method of preventing cell death, said method comprising administering a polypeptide of claim 12 to a subject.

19. (Amended) A method of preventing cell death, wherein said method comprises administering to a patient the protein of claim 13 at a therapeutically effective dose.

REMARKS

The invention includes mutant Ced-3, ICE, and NEDD-2 proteins, such as proteins having inactivating amino acid substitutions. These proteins are useful for the prevention of cell death.

Support for the Amendments

Claims 1-4, 6-16, 18, and 19 have been amended to correct informalities, antecedent basis, and more precisely define the invention. In particular, amended claims 1 and 9-16 now more precisely recite the position of the amino acid substitution and distinctly claim the subject matter which applicants regard as the invention. Amended claim 12 also clarifies that the specified amino acids are the residues from the corresponding naturally-occurring proteins that are mutated in the recited proteins.

Claim 1 has also been amended to further clarify that the claimed protein is a "protein product of a gene which has identity with the ced-3 and ICE genes." Support for this amendment can be found in the specification at page 3, lines 8-11; page 41, lines 13-24; and page 51, lines 17-20. Claim 8 has been amended to recite proteins that have an amino acid substitution in the ICE sequence relative to the wild-type amino acid sequence shown in Figure 6A. Support for this amendment can be found in the specification at page 3, lines 22-26, and page 41, line 2 to page 42, line 3. Claims 6-8 have been amended to further clarify that the claimed proteins have a deletion of the inhibitory amino terminal portion of the wild-type sequence. Support for this amendment

can be found at page 5, lines 18-19.

Claims 1, 4, 8, 12, and 13 have been amended to recite proteins having a mutation relative to SEQ ID NO: 30, the naturally-occurring amino acid sequence of human ICE from Fig. 6A, and claim 6, 7, 12, and 13 have been amended to recite proteins having a mutation relative to SEQ ID NO: 29, the naturally-occurring amino acid sequence of *C. elegans* Ced-3 from Fig. 6A (as disclosed, for example, on pages 27- 31 of the specification).

Claims 9-13 and 16 have been amended to recite NEDD-2 mutations relative to SEQ ID NO: 28, the amino acid sequence of murine NEDD-2 in Fig. 2 of Fernandes-Alnemri *et al., J. Biol. Chem.* 269:30761, 1994, a copy of which is enclosed. As indicated in the attached Declaration of Dr. Bieker-Brady, this publication is incorporated by reference in the present application as a part of the definition of asp-asc proteases of the invention (page 32, lines 7-10, of the specification). As the amino acid sequence of SEQ ID NO: 28 is the same as that of the sequence incorporated by reference, this amendment contains no new matter. In particular, as indicated in MPEP § 2163.07(b), information incorporated by reference is part of the application as filed and amending the application to contain the actual text of the incorporated matter is not new matter.

Applicants note that the amino acid numbering for NEDD-2 using in the specification is based on the numbering of the partial NEDD-2 amino acid sequence disclosed by Kumar *et al.* (*Biochem. Biophys. Res. Comm. 185*(3):1155-1161, 1992) and found in SEQ ID NOs: 13 and 16 (see for example, page 51, lines 20-23, of the specification). One skilled in the art would appreciate which residues of the 451 amino acid full-length NEDD-2 sequence incorporated by reference to Fernandes-Alnemri (SEQ ID NO: 28) correspond to the residues in the 171 amino acid partial NEDD-2 sequence of Kumar (SEQ ID NO: 13). For example, as illustrated in the sequence alignment of Exhibit A in the Declaration, residues 43, 44, 46, 67, 82, 97, 102, 103, 108, 122, 158, 163, and 166 of SEQ ID NO: 13 recited in claim 12 as filed correspond to residues 323, 324, 326, 327, 362, 377, 282, 383, 386, 388, 402, 438, 443, and 446,

respectively, of SEQ ID NO: 28 recited in amended claim 12. Similarly, residue 117 of SEQ ID NO: 26 recited in claim 9 as filed corresponds to residue 397 of SEQ ID NO: 28 recited in amended claim 9. As one skilled in the art would appreciate that proteins having the mutations relative to SEQ ID NO: 28 that are recited in amended claims 9-13 and 16 contain the NEDD-2 mutations disclosed in applicants' specification, this amendment contains no new matter.

Claims 10, 11, 13, and 16 have also been amended to recite NEDD-2 proteins with mutations in the active site cysteine of SEQ ID NO: 28. As stated on page 6, line 1 and page 29, lines 16-23, NEDD-2 proteins in which the catalytic cysteine is mutated are useful for inhibiting cell death. As illustrated in the sequence alignment of Exhibit B in the Declaration, the catalytic cysteine 303 of the NEDD-2 sequence of Genbank accession number P29594 corresponds to cysteine 319 of the NEDD-2 sequence of SEQ ID NO: 28, which has additional amino acids because of an earlier translation start site. Thus, one skilled in the art would appreciate that the cysteine 319 of the NEDD-2 sequence of SEQ ID NO: 28 corresponds to the active site cysteine of NEDD-2.

No new matter is added by these amendments.

Conclusion

In summary, applicants submit that the claims are now in condition for allowance, and such action is respectfully requested. A marked-up version indicating the amendment made to the claims, as required by 37 C.F.R. § 1.121(c)(1)(ii), is enclosed. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 16, 2002

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	H. Robert Horvitz et al.	Art Unit:	Not yet assigned
Serial No.:	09/888,243	Examiner:	Not yet assigned
Filed:	June 22, 2001	Customer No.:	21559
Title:	RELATEDNESS OF HUMAN INTERLEUKIN-1β CONVERTASE GENE TO A *C. ELEGANS* CELL DEATH GENE, INHIBITORY PORTIONS OF THESE GENES AND USES THEREFOR		

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Versions with Markings to Show Changes Made

In the claims:

Marked-up versions of claims 1-4, 6-16, 18, and 19 are presented below.

1. (Amended) An isolated protein, consisting of a protein product of a gene which [is structurally related to] has identity with the *ced-3* and Interleukin-1β convertase (ICE) genes, said isolated protein having an alteration in the amino acid sequence of the product of a gene which [is structurally related to] has identity with the *ced-3* and ICE genes, said alteration corresponding to an amino acid substitution [alteration] in the sequence of SEQ ID NO: 4 or 30 selected from the group consisting of:

i) [L to] F at amino acid 26;

ii) [G to] R at amino acid 65;

iv) [G to] S at amino acid 287;

v) truncation of said protein after amino acid 323;

vi) truncation of said protein after amino acid 339;

vii) [A to] V at amino acid 361;

viii) [E to] K at amino acid 390; and

ix) [T to] F at amino acid 393.

2. (Amended) The protein [protease] of claim [Claim] 1, which is a protease cleaving [cleaves] after aspartate residues.

3. (Amended) The protein [protease] of claim [Claim] 1, which is a cysteine protease.

4. (Amended) An isolated ICE polypeptide (SEQ ID NO: 4 or 30) having an alteration which reduces the proteolytic activity of the [enzyme] protein, wherein said alteration is an amino acid substitution selected from the group consisting of:

a) [L to] F at amino acid 26;

b) [G to] R at amino acid 65;

c) [G to] S at amino acid 287;

d) truncation of said polypeptide after amino acid 323;

e) truncation of said polypeptide after amino acid 339;

f) [A to] V at amino acid 361;

g) [E to] K at amino acid 390; and

h) [T to] F at amino acid 393.

6. (Amended) A constitutively activated cell death protein comprising an amino acid sequence having deletions of the inhibitory amino-terminal portions, said sequence

comprising a portion of the Ced-3 protein shown in SEQ ID NO: 2 of Figure 6A or SEQ ID NO: 29, said portion selected from the group consisting of:

a) the amino acids from [approximately] 150 to 503 (SEQ ID NO:[_] 20);

b) the amino acids from [approximately] 373 to 503 (SEQ ID NO: 21); and

c) the amino acids from [approximately] 150 to 372 (SEQ ID NO: 22).

7. (Amended) The [constitutively activated cell death] protein of claim 6, further comprising a subportion of the region of Ced-3 from amino acids 1 to 149, as shown in SEQ ID NO: 2 of Figure 6A or SEQ ID NO: 29, said subportion enhancing the proteolytic activity of the protein.

8. (Amended) A constitutively activated cell death protein comprising an amino acid sequence having deletions of the inhibitory amino-terminal portions, said protein

(a) having a substitution in the amino acid sequence ICE relative to the ICE sequence shown in Figure 6A (SEQ ID NO: 4) or SEQ ID NO: 30; and

(b) comprising a portion of the ICE sequence in SEQ ID NO: 4 or 30, said portion [said sequence] selected from the group consisting of:

i) the amino acids from [approximately] 111 to 404 (SEQ ID NO: 23);

ii) the amino acids from [approximately] 298 to 404 (SEQ ID NO: 24); and

iii) the amino acids from [approximately] 111 to 297 (SEQ ID NO: 25).

9. (Amended) [The] An isolated protein [which is] having an amino acid alteration in the NEDD-2 protein (SEQ ID NO: 28 [26]), said alteration [having] being an alteration which inactivates [the] said protein, wherein said alteration is an amino acid substitution of A to V at amino acid 397 [117], relative to the sequence of SEQ ID NO: 28.

10. (Amended) [The] An isolated protein having an amino acid alteration in the NEDD-2 protein (SEQ ID NO: 28, said alteration being an alteration which inactivates

said protein, [of claim 9] wherein said alteration is an amino acid substitution of C to A at amino acid 319 [303], relative to the sequence of SEQ ID NO: 28.

11. (Amended) [The] An isolated protein having an amino acid alteration in the NEDD-2 protein (SEQ ID NO: 28), said alteration being an alteration which inactivates said protein, [of claim 9] wherein said alteration is an amino acid substitution of C to S at amino acid 319 [303], relative to the sequence of SEQ ID NO: 28.

12. (Amended) An isolated [Isolated] protein which is selected from the group consisting of Ced-3 (SEQ ID NO: 2 or 29), ICE (SEQ ID NO: 4 or 30), and NEDD-2 (SEQ ID NO: 28 [13]), said protein having an alteration [at a conserved amino acid corresponding to an amino acid of the Ced-3 protein (SEQ ID NO. 2 or 29)] selected from the group consisting of a substitution in amino acid:

a) Ser 183 of Ced-3 [Ser 183] or Ser 126 of ICE [Ser 126];

b) Met 234 of Ced-3 [Met 234];

c) Arg 242 of Ced-3 [Arg 242];

d) Leu 246 of Ced-3 [Leu 246] or Leu 166 of ICE [Leu 166];

e) Ile 247 of Ced-3 [Ile 247] or Ile 167 of ICE [Ile 167];

f) Ile 248 of Ced-3 [Ile 248] or Ile 168 of ICE[Ile 168];

g) Asn 250 of Ced-3 [Asn 250] or Asn 170 of ICE [Asn 170];

h) Phe 253 of Ced-3 [Phe 253] or Phe 173 of ICE [Phe 173];

i) Arg 259 of Ced-3 [Arg 259] or Arg 179 of ICE [Arg 179];

j) Gly 261 of Ced-3 [Gly 261] or Gly181 of ICE [Gly 181];

k) Asp 265 of Ced-3 [Asp 265] or Asp 185 of ICE [Asp 185];

l) Gly 277 of Ced-3 [Gly 277] or Gly 197 of ICE [Gly 197];

m) Tyr 278 of Ced-3 [Tyr 278] or Tyr 198 of ICE [Tyr 198];

n) Val 280 of Ced-3 [Val 280] or Val 200 of ICE [Val 200];

o) Lys 283 of Ced-3 [Lys 283] or Lys 203 of ICE [Lys 203];

p) Asn 285 of Ced-3 [Asn 285] or Asn 205 of ICE [Asn 205];

q) Leu 286 of Ced-3 [Leu 286] or Leu 206 of ICE [Leu 206];

r) Thr 287 of Ced-3 [Thr 287] or Thr 207 of ICE [Thr 207];

s) Met 291 of Ced-3 [Met 291] or Met 211 of ICE [Met 211];

t) Phe 298 of Ced-3 [Phe 298] or Phe 218 of ICE [Phe 218];

u) His 304 of Ced-3 [His 304] or His 244 of ICE [His 224];

v) Asp 306 of Ced-3 [Asp 306] or Asp 228 of ICE [Asp 228];

w) Ser 307 of Ced-3 [Ser 307,] or Ser 229 of ICE [Ser 229, or NEDD-2 Ser 16];

x) Leu 310 of Ced-3 [Leu 310,] or Leu 232 of ICE [Leu 232, or NEDD-2 Val 19];

y) Val 311 of Ced-3 [Val 311,] or Val 233 of ICE [Val 233, or NEDD-2 Val 20];

z) Ser 314 of Ced-3 [Ser 314] or Ser 236 of ICE [Ser 236];

aa) His 315 of Ced-3 [His 315] or His 237 of ICE [His 237];

bb) Gly 316 of Ced-3 [Gly 316] or Gly 238 of ICE [Gly 238];

cc) Ile 321 of Ced-3 [Ile 321,] or Ile 243 of ICE [Ile 243, or NEDD-2 Leu 23];

dd) Gly 323 of Ced-3 [Gly 323,] or Gly 245 of ICE [Gly 245, or NEDD-2 Asp 25];

ee) Ile 334 of Ced-3 [Ile 334,] or Ile 261 of ICE [Ile 261, or NEDD-2 Phe 31];

ff) Asn 339 of Ced-3 [Asn 339] or Asn 226 of ICE [Asn 266];

gg) Pro 344 of Ced-3 [Pro 344] or Pro 271 of ICE [Pro 271];

hh) Leu 346 of Ced-3 [Leu 346] or Leu 273 of ICE [Leu 273];

ii) Lys 349 of Ced-3 [Lys 349] or Lys 276 of ICE [Lys 276];

jj) Pro 350 of Ced-3 [Pro 350,] or Pro 277 of ICE [Pro 277, or NEDD-2 Pro 37];

kk) Lys 351 of Ced-3 [Lys 351] or Lys 278 of ICE [Lys 278];

ll) Gln 356 of Ced-3 [Gln 356], Gln 283 of ICE [Gln 283], or Glu 323 of

NEDD-2 [Glu 43];

mm) Ala 357 of Ced-3 [Ala 357], Ala 284 of ICE [Ala 284], or Thr 324 of NEDD-2 [Thr 44];

nn) Cys 358 of Ced-3 [Cys 358] or Cys 285 of ICE [Cys 285];

oo) Arg 359 of Ced-3 [Arg 359], Arg 286 of ICE [Arg 286], or Arg 326 of NEDD-2 [Arg 46];

pp) Gly 360 of Ced-3 [Gly 360], Gly 287 of ICE [Gly 287], or Gly 327 of NEDD-2 [Gly 47];

qq) Asp 371 of Ced-3 [Asp 371] or Asp 297 of ICE [Asp 297];

rr) Asp 414 of Ced-3 [Asp 414], Asp 326 of ICE [Asp 326], or Asp 362 of NEDD-2 [Asp 82];

ss) Arg 429 of Ced-3 [Arg 429], Arg 341 of ICE [Arg 341], or Arg 377 of NEDD-2 [Arg 97];

tt) Gly 434 of Ced-3 [Gly 434], Gly 346 of ICE [Gly 346], or Gly 382 of NEDD-2 [Gly 102];

uu) Ser 435 of Ced-3 [Ser 435], Ser 347 of ICE [Ser 347], or Ser 383 of NEDD-2 [Ser 103];

vv) Ile 438 of Ced-3 [Ile 438], Ile 350 of ICE [Ile 350], Ile 386 of NEDD-2 [Ile 106];

ww) Ala 449 of Ced-3 [Ala 449], Ala 361 of ICE [Ala 361], or Ala 388 of NEDD-2 [Ala 108];

xx) Val 454 of Ced-3 [Val 454], Val 366 of ICE [Val 366], or Val 402 of NEDD-2 [Val 123];

yy) Leu 488 of Ced-3 [Leu 488], Leu 394 of ICE [Leu 394], or Leu 438 of NEDD-2 [Leu 158];

zz) Tyr 493 of Ced-3 [Tyr 493], Tyr 399 of ICE [Tyr 399], or Tyr 443 of NEDD-2 [Tyr 163]; and

aaa) Pro 496 of Ced-3 [Pro 496], Pro 402 of ICE [Pro 402], or Pro 446 of

NEDD-2 [Pro 166].

13. (Amended) An isolated [Isolated] protein selected from the group consisting of Ced-3 (SEQ ID NO: 2 or 29), NEDD-2 protein (SEQ ID NO: 28), and ICE (SEQ ID NO: 4 or 30), said protein having an amino acid alteration, wherein said alteration [in an amino acid corresponding to] is at Cys 358 of Ced-3, Cys 319 of NEDD-2, or Cys 285 of ICE.

14. (Amended) The isolated protein of claim 13, wherein said amino acid alteration at Cys 358 of Ced-3 or Cys 285 of ICE is a Cys to Ala alteration.

15. (Amended) The isolated protein of claim 13, wherein said protein is ICE and said amino acid alteration is at conserved amino acid Cys 285 of said ICE.

16. (Amended) The isolated protein of claim 13, wherein said protein is NEDD-2 and said alteration is at conserved amino acid amino acid 319 [303] of said NEDD-2 (SEQ ID NO: 28).

18. (Amended) A method of preventing cell death, said method comprising administering a polypeptide of claim 12 to a subject.

19. (Amended) A [The] method of preventing cell death, wherein said method comprises administering [is] to a patient the protein of claim 13 [polypeptide is provided] at a therapeutically effective dose.